

Page 32, delete the paragraphs spanning lines 1-5, and insert the following therefor:

--The genomic organization of murine KARAP is shown in Figure 18. We also obtained the cDNA sequence and hence the protein sequence of murine KARAP of the 129 line from these data. This cDNA sequence (SEQ ID no. 27) and the protein sequence (SEQ ID no. 28) are shown in Figure 19.

The protein sequence translated in this way is (SEQ ID NO:43):--

Insert the attached Sequence Listing, in place of the copy of the Sequence Listing filed October 29, 1999.

#### **REMARKS**

Reconsideration is requested.

A copy of the Notice to Comply and Error Report received with the Office Action of February 26, 2002, are attached.

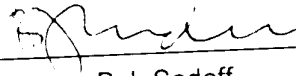
The specification has been amended to include the attached Sequence Listing and corresponding Sequence identifiers. No new matter has been added. The attached paper and computer readable copies of the Sequence Listing are the same. A separate Statement to this effect is attached.

An early and favorable Action on the merits is requested.

Respectfully submitted,

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By: \_\_\_\_\_



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**MARKED-UP COPY OF AMENDMENTS TO SPECIFICATION**

**IN THE SPECIFICATION:**

Amend the specification as follows:

Page 35, delete the paragraphs spanning lines 35-39 and insert the following therefor:

-- - Figure 8 shows the nucleotide sequence (residues 138 to 398 of SEQ ID NO: 1) (comprised between the excluded leader sequence and the stop codon) and the amino acids sequence of a KARAP polypeptide according to the invention (mature protein, SEQ ID no. 2), and

- Figure 9 shows the alignment of the ITAMs (SEQ ID NOS 32-41, respectively in order of appearance) and of the ITAM of a KARAP polypeptide (SEQ ID NO: 42) according to the invention, --

Page 16, delete the paragraphs spanning lines 7-10, and insert the following therefor:

-- - Figure 15 shows the alignment of the cDNA sequences of the EST's AA242315 (SEQ ID NO:6), AA734769 (SEQ ID NO:7), W88159 (SEQ ID NO:8), AA098506 (SEQ ID NO:9) and W41142 (SEQ ID NO:6), and the resulting consensus sequence (SEQ ID no. 16; KARAP consensus cDNA of mouse C57Bl/6),

- Figure 16 represents the alignment of the protein sequences of the EST's AA242315(SEQ ID NO:11), AA734769 (SEQ ID NO:12), W88159 (SEQ ID NO:13), AA098506 (SEQ ID NO:14) and W41142 (SEQ ID NO:15), and the resulting consensus sequence (SEQ ID no. 17; KARAP consensus protein of mouse C57Bl/6),--

Page 16, delete the paragraph spanning lines 21-23 and insert the following therefor:

-- - Figure 20 represents from top to bottom the genomic organization of the KARAP gene of a mouse of line 129, the corresponding protein sequence (SEQ ID NO:44), and the nature of the different regions of this protein,--

Delete the paragraph spanning page 27, line 18 to page 28, line 24 and insert the following therefor:

--By combining these two biological data processing approaches and after having successively determined the leader, transmembrane, intracytoplasmic and extracytoplasmic regions of the candidate molecules with the aid of hydrophobicity profiles (Genworks and DNA Strider programs), we obtained a large number of sequences potentially corresponding to that of KARAP. Among these sequences, the one corresponding to accession number AA242315 in Genbank appeared to us to be the sequence of the murine KARAP gene (SEQ ID no. 1, C57Bl/6 murine cDNA). Figure no. 7 shows the DNA sequence (SEQ ID no. 1, cDNA) of a KARAP polypeptide according to the invention; this sequence corresponds to the sequence of the murine KARAP gene. In fact, translation of the nucleotide sequence gives an open reading

frame of 396 nucleotides (SEQ ID no. 2). This result is illustrated in Figure no. 8, which shows that part of the nucleotide sequence of the KARAP gene (SEQ ID no. 1) which is between the leader sequence (excluded) and the stop codon, and which also shows, underneath this nucleotide sequence, the corresponding amino acid sequence (1-letter code) (SEQ ID no. 2, 3-letter code), i.e. the amino acid sequence of the mature murine KARAP according to the invention (SEQ ID no. 2). Standard analysis of this sequence predicts a mature protein of 87 amino acids (molecular weight of 9.6 kDa), an extracytoplasmic part of 16 amino acids (Q<sub>1</sub>-G<sub>16</sub>), a transmembrane part of 24 amino acids (V<sub>17</sub>-G<sub>40</sub>) and an intracytoplasmic part of 47 amino acids (R<sub>41</sub>-R<sub>87</sub>). According to our search strategy, the extracytoplasmic part comprises at least one cysteine amino acid (in fact two, C<sub>8</sub> and C<sub>10</sub>), a transmembrane amino acid (D<sub>25</sub>) and an intracytoplasmic ITAM (Y<sub>65</sub>QELQGQRHEVY<sub>76</sub>SDL). Figure 9 (SEQ ID NO:42) illustrates the comparisons which can be made by aligning sequences between the ITAM polypeptides described previously and the polypeptide according to the invention possessing one (or more) ITAMs, and indicates the resulting consensus ITAM sequence: Figure 9 shows the alignment of the ITAMs of ITAM polypeptides (six CD3, one Ig $\alpha$ , one Ig $\beta$ , Fc $\epsilon$ R1 $\gamma$  and Fc $\epsilon$ R1 $\beta$ ) and an ITAM of the murine KARAP polypeptide (SEQ ID no. 2) identified above according to the invention (labelled "KARAP" in said Figure 9). On the basis of this comparison with the ITAMs described previously (Figure 9), we were able to envisage the association of the phosphorylated KARAPs with tyrosine kinase proteins containing SH2 groups in tandem (proteins such as ZAP-70 and p72Syk). The association of KARAPs with recombinant fusion proteins corresponding to the SH2 groups of ZAP-70 (preparation described in: Olcese L., Lang

P., Vély F., Cambiaggi A., Marguet D., Bléry M., Hippen K.L., Biassoni R., Moretta A., Moretta L., Cambier J.C., Vivier E., 1996, J. Immunol., 156, 4531-4534) was verified *in vitro*: these experiments were carried out as described in Figure 3A, lane 3, but the cell lysates were adsorbed by the recombinant fusion protein corresponding to the SH2 groups of ZAP-70 instead of the anti-CD158 antibody. Thus KARAP is a novel ITAM transmembrane molecule which associates with KARs and which, in a phosphorylated tyrosine form, associates with ZAP-70. KARAP is therefore a novel transducing element of T and NK lymphocytes. It is possible that KARAP or KARAP analogues also associate with the activatory isoforms of ITIM receptors and serve in these multimolecular complexes as subunits for transducing the signals emitted when the receptor is taken up.--

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